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INTRODUCTION

The relationship of extraembryonic membrane function to the progression of cancers, specifically cancers of the breast, is an understudied area of research. By understanding the factors, which control the molecular architecture of the placenta and other fetal membranes, we hope to shed light on mechanisms by which placental function is established. The initial goal of this research had been to characterize the role of the gene *mPlab* in mice conditionally null for the gene. *mPlab* is a member of the TGF-β superfamily of transforming growth factors, and had been previously demonstrated to be expressed at highest levels in the developing placenta. Midway through our research, we discovered that another laboratory had published the phenotype of the *mPlab* deficient mice (1). In light of this, we were forced to abandon our research on *mPlab* and find other areas of fetal membrane research to explore. In this report, we detail the completed work on our revised proposal. Additionally, the DOD had indicated that it would not be willing to fund research requiring work with non-human primate materials as described in the annual report dated June 2001. In order to alleviate these concerns, we submit a modification of the new research proposal to the reviewing boards.

BODY.

The goal of our original project was to characterize the loss of function of MPLAB in transgenic mice. In the May 2000 issue of Molecular and Cellular Biology, Se-Jin Lee's laboratory at Johns Hopkins University School of Medicine reported successful generation of a null allele of this gene and the resultant phenotype (1). With regards to the Statement of Work supplied with our original DOD funding application, we had successfully completed approximately half of the work contracted with the Department of Defense. We had independently cloned and determined the sequence of *mPlab*(*gdf-15*), constructed a targeting vector to generate a conditionally null allele of the gene, and generated transgenic embryonic stem cells from that vector.

Our research over the intervening time shifted to the development of an entirely new set of specific aims which seeks to test the mouse model as a legitimate model for primate embryology, especially with regard to the early establishment of the extra-embryonic membranes. Extraembryonic membrane biology was also the focus of the *mPlab* (*gdf-15*) study.

The basic premise of this research is that we, as a mouse research laboratory, use the mouse as a model to study early embryonic development for the ultimate purpose of understanding development in other mammalian species, specifically the human. The natural history of the mouse, as well as the availability of multiple congenic lineages, have made it particularly attractive as a genetic tool for research in numerous biological phenomena. The underlying assumption is, however, that the mouse closely models the biology of other animal systems. The reality of the matter is that there are numerous documented cases where research in the mouse or human does not faithfully predict the biology of the reciprocal species (2).

This is probably most carefully studied in pharmacokinetic literature, but is increasingly evident in other fields. A review of recent literature reveals mouse-human differences in the

biologies of menopause (3), breast cancer (4,5), ovarian function (6), and wound repair (7) among others (8,9,10).

In my specific interest of developmental biology, it is known for example that many of the functions carried out by human placental estrogens, are performed in mice by a wholly dissimilar class of molecules, the prolactins. The mouse placenta is, in fact, incapable of producing aromatase, an enzyme necessary for production of estrogens. Promoter studies of the human aromatase gene, however, have shown that elements of the human aromatase promoter can drive reporter gene expression in the murine placenta (11). This suggests that although the mature organ biology is quite different, the underlying molecular architecture of the two species may still be quite conserved. There exists, therefore, a class of placental research for which the tools available to murine biologists may provide significant advances in understanding of primate placental development and function. For these same reasons, however, it is necessary for mouse researchers to test the fidelity of their models in other species in order to assess where the limitations of the mouse may lie in any given field of research.

Here we present a project centered on the observation that primate and murine gastrulae display markedly disparate morphologies. This, of course, leads to the question of whether the differing morphologies are of similar molecular determination, or whether they are uniquely derived with respect to one another.

Whereas the initial description of the new proposal was to define primate embryonic structures in molecular terms by examining expression patterns of murine molecular markers in primate gastrula-staged embryos, we have modified this step in accordance with the concern of our reviewing board. We are currently generating a new interspecific chimera technology to establish and model primate (or other xenotypic mammalian embryos) within the mouse, using tetraploid mouse cells to substitute early placental functions of the xenotypic embryo. We are, in essence, asking the question: "Can the extraembryonic tissues of the mouse support the early development of xenotypic embryos?" This technology will take advantage of previously established embryonic stem cell lines of the Rhesus macaque (12), and will not require the procurement of embryonic tissue from non-human primates as detailed in the previous annual report. Furthermore, a pilot study is underway to test this technology in rat:mouse interspecific chimeras prior to development of the non-human primate:mouse technologies. These pilot studies are based on a series of 30 year old papers in which successful embryonic chimeras of rat and mouse embryos were produced. These chimeras were generally created by either morula aggregation or by injection of rat inner cell masses into mouse blastulae. Using species specific antibodies, it was seen that the rat cells are capable of colonizing epiblast and trophectoderm tissues, but were twice as abundant in the endodermal lineages (13). In another study, which included 7.5 day embryos, rat contribution to mesoderm was seen (14). Though one paper reported the ability to culture these chimeras to 9.5 days of development (13), it was later found that a strong selection against rat cells had occurred and that these embryos consisted of little or

no rat tissue (15). Such negative selection was blamed on maternal immune response, and was also seen in more closely related interspecific chimeras. In *M. caroli:M. musculus* ICM injection chimeras, this negative selection could be overcome by ensuring that the recipient blastocyst was of the same strain as the host mother (16). This suggests that trophoblast tissue is responsible for the protection of the interspecific tissue. The alternative explanation is that a heterogeneous trophoblast simply does not function at a level required for maintaining the life of the conceptus. Despite the problems, these papers proved that rat and mouse chimeras were capable of developing in concert to produce viable gastrulae.

Today, tetraploid embryos can be used in combination with embryonic stem cells to produce embryos of 100% ES cell derivation inside a trophoblast shell of 100% host embryo derivation (17). We would like to ask the question "Can the trophoblast tissue of a mouse support to early development of a xenotypic embryo?". Our hypothesis is that a tetraploid mouse trophoblast will be able to shield the developing xenotypic embryo from the maternal immune system and provide a suitable surrogate placenta for the early development of the xenotypic embryo.

Due to relative phylogenetic distance and the existence of a large body of literature on rat:mouse chimeras, our primary goal is to produce interfamilial chimeras between rat and mouse using tetraploid mouse blastocysts. Pending successful production of these chimeras it is envisioned that we will apply this technology to the production of chimeras from tetraploid mouse embryos and embryonic stem cells of the rhesus macaque

Rat embryonic stem cells are available, but due to difficulties several labs have had maintaining these cells we have opted to isolate inner cell masses from rat blastocysts for injection. The rat blastocysts we will use are being provided by Dr. Robert Hammer of the UT Southwestern Medical Center. Dr. James Thompson of University of Wisconsin Primate research center has generously promised us use of Rhesus macaque embryonic stem cells for this research.

To date, G.E. has made several successful practice chimeras of mouse:mouse type and is now producing chimeras using tetraploid blastocysts by blastocyst injection and aggregation chimera technology. After initial difficulties establishing the electrofusion technique in our lab, G.E. has improved tetraploid embryo production to a point that 70% of treated two cell embryos develop to blastocyst stage.

We have also begun examining the development of transferred tetraploid blastocysts in pseudopregnant mice as a control for the later interspecific chimera experiments. Preliminary evidence suggests that the tetraploid embryos of Swiss mice are incapable of forming gastrula on their own and arrest at perimplantation.

Additionally, while reviewing pertinent literature it became apparent that the studies initially describing embryonic stem cell growth in tetraploid blastocysts had only been performed on embryos older than E8.5. Since gastrulation occurs prior to E8.5 it is essential to describe 4N:ES cell chimera development at earlier stages to insure that the distributions of tetraploid and ES cells are truly segregated as described in later development. These embryos are currently under production and preliminary data sets are expected in the first week of July.

KEY RESEARCH ACCOMPLISHMENTS

- Cloning and sequencing of the *mPlab* gene
- Creation of a targeting vector for the production of a conditionally null allele of *mPlab*.
- Establishment of an ES cell lines putatively heterozygous for the *mPlab* conditionally null allele.
- Establishment of tetraploid production, culture, and aggregation chimera technologies and in our laboratory
- Analysis of gastrulation defect in control tetraploid mouse embryos of Swiss strain.

REPORTABLE OUTCOMES

There are currently no reportable outcomes for DAMD 17-1-0311, as described in the "training reporting requirements" http://mrmc-www.army.mil/rrptraining.asp

CONCLUSIONS

An unfortunate consequence of the large scale and fast-pace of scientific research is that often multiple laboratories are working on closely related projects, and may be unaware of the other's efforts. This was the case with our research on the *mPlab* gene. In late December, 2000, we became aware that our effort to construct null alleles of the murine *mPlab* gene was a duplication of published work in another laboratory. It was apparent that continued research on mPlab would not be a productive endeavor for either the laboratory or the education of the graduate student (G.E.) performing the research. As such we designed a new project, submitted in the previous annual report, outlining a research program intended to test the limitations of the mouse system as a model for primate early development and disease. In this annual review we address concerns of the reviewing board and propose an alternative technology for studying primate embryonic development using only pre-existing non-human primate embryonic stem cells. This research is of crucial step in the establishment of mouse models as numerous very basic differences between the biologies of mice and primates are appearing in the literature. Cancer is diagnosed during 1 in 1000 pregnancies. As more and more women delay their first pregnancies into later years, the incidence of cancer diagnosis during pregnancy is expected to increase. Our ability to bring the power of mouse research to bear against these questions rests, therefore, on our knowledge of the limitations to which the mouse can serve as a model for these disease.

REFERENCES

1. E.C. Hsiao, et al. Characterization of growth-differentiation factor 15, a transforming growth factor beta superfamily member induced following liver injury. *Mol Cell Biol* **20** 3742-51 (2000).
2. A. Wynshaw-Boris. Model mice and human disease. *Nat Genet* **13**, 259-260 (1996).
3. F.L. Bellino. Nonprimate animal models of menopause: workshop report. *Menopause* **7**, 14-24 (2000).
4. A.D. Cardiff. Validity of mouse mammary tumour models for human breast cancer: comparative pathology. *Microsc Res Tech* **52**, 224-230 (2001).
5. S. Nandi. Hormones and mammary carcinogenesis in mice, rats, and humans: a unifying hypothesis. *Proc Natl Acad Sci U S A.* **92**, 3650-3657 (1995).
6. B.S. Dunbar et al. The ovary as an immune target. *J Soc Gynecol Investig* 8 (1 Suppl Proceedings):S43-8 2001
7. A.B. Borisov. Regeneration of skeletal and cardiac muscle in mammals: do nonprimate models resemble human pathology? *Wound Repair Regen* **7**, 26-35. (1999).
8. C.M. Disteche. Escape from X inactivation in human and mouse. *Trends Genet* **11**, 17-22 (1995).
9. W.E. Wright and J.W. Shay. Telomere dynamics in cancer progression and prevention: fundamental differences in human and mouse telomere biology. *Nat Med* **6**, 849-851 (2000).
10. S.V. Prasad et al. Structure and function of the proteins of the mammalian Zona pellucida. *Cells Tissues Organs* **166**, 148-164 (2000).
11. A. Kamat, K. H. Graves, M. E. Smith, J. A. Richardson, C. R. Mendelson. A 500-bp region, approximately 40 kb upstream of the human CYP19.*Proc Natl Acad Sci U S A* **96**, 4575-80 (1999).
12. Thomson, JA., Kalishman, J. et al. (1995) "Isolation of a Primate Embryonic Stem Cell line" *Proc Natl Acad Sci U S A* **92(17)**, 7844-7848
13. Gardner, R. L. and M. H. Johnson (1973). "Investigation of Early Mammalian Development using Interspecific Chimeras between Rat and mouse." Nature New Biology **246**(151): 86-89.
14. Rossant, J. (1976). "Investigation of inner cell mass determination by aggregation of isolated rat inner cell masses with mouse morulae." J Embryol Exp Morphol **36**(1): 163-74.
15. Gardner, R. L. and M. H. Johnson (1975). "Investigation of cellular interaction and deployment in the early mammalian embryo using interspecific chimaeras between the rat and mouse." Ciba Found Symp **0**(29): 183-200.

16. Rossant, J., B. A. Croy, et al. (1982). "Interspecific chimeras in mammals: a new experimental system." J Anim Sci **55**(5): 1241-8.
17.Nagy, A., E. Gocza, et al. (1990). "Embryonic stem cells alone are able to support fetal development in the mouse." Development **110**(3): 815-21.

APPENDICES